REMARKS

Applicants have amended claims 1, 6, 20, 25, 41, 42 and 44 to specify that the pro-peptide is from chymosin. Applicants have also amended claims 1 and 13-16 in order to specify that the autocatalytically maturing zymogen added in step c) is an aspartic protease. It is believed that the present amendment addresses the issues raised in the Advisory Action that was mailed on December 19, 2002 as well as the issues raised in the telephone interview on January 30, 2003.

In the Advisory Action, it is noted that all of the previous objections have been withdrawn with the exception of the rejection of claims 1, 4-20, 23-26, 28-30 and 41-44 under 35 USC §112, first paragraph as lacking enablement. It appears that the Examiner has two main concerns with respect to enablement. The first is the scope of the pro-peptide which can be from any aspartic protease and the second is the scope of the mature zymogen which is added in step c) of claim 1. Each of these are addressed in turn below.

With respect to the first issue, the Examiner comments that only one aspartic protease, chymosin, is supported by a working example. Applicants submit that undue experimentation would not be required by one of skill in the art in order to enable the full scope of the claims with respect to using a pro-peptide from any aspartic protease. Aspartic proteases are a group of zymogens with both a similar protein structure and mechanism of action. In particular, all aspartic proteases have the following characteristics:

- They all have an active site that contains two invariant aspartate residues (Davies, *Ann Rev Bioph Chem* 19:189-215, 1990), positioned in close

geometric proximity, within a substrate cleft between bilobal N- and C-terminal domains (Foltmann, *Biol Chem Hoppe-Seyler* 369:311-314, 1988).

- They are all activated by the proteolytic removal of the N-terminal pro-peptide, usually 44-50 amino acids long, following synthesis (Foltmann, *Biol Chem Hoppe-Seyler* 369:311-314, 1988; Boel et al., *Proteins: Structure, Function, and Genetics* 1: 363-369, 1986).
- They are all inhibited by pepstatin A (Umezawa et al., *J Antibiotics* 23:259-262, 1970) and are optimally active in an acidic pH ranging from 1-5. (László, *CRC Press Inc.*, Boca Raton, Florida, USA. pp 157-182, 1989).
- They all have a catalytic mechanism which is characterized by nucleophilic attack of the scissile peptide bond by an activated water molecule and does not involve a covalent intermediate (Davies, *Ann Rev Bioph Chem* 19:189-215, 1990). At low pH one aspartate of the catalytic dyad is ionized and the second aspartate is protonated with a water molecule polarized between the aspartyl residues by hydrogen bonding. The oxygen of the water molecule engages in nucleophilic attack of the substrate carbonyl carbon of the peptide bond resulting in a tetrahedral carbon intermediate. The peptide bond is ultimately cleaved following donation of hydrogen from an aspartate to substrate nitrogen.

Therefore, aspartic proteases display the same characteristics with respect to structure, enzyme activation, and the catalytic mechanism of peptide cleavage. As a result, we submit that one of skill in the art would readily expect that a pro-peptide from any aspartic protease would be useful in the present invention.

Notwithstanding the above, Applicants have amended the claims to specify that the pro-peptide is from chymosin. This amendment is made without prejudice and solely for the purposes of advancing prosecution of this application.

The second issue that the Examiner raises is that undue experimentation would be required in order to determine which autocatalytically maturing zymogen could be added for cleavage of the pro-peptide. In response, the claims have been amended in order to specify that the zymogen added in step c) of claim 1 is an aspartic protease that can cleave the chymosin pro-peptide sequence. Applicants have provided four examples of aspartic proteases that can cleave the chymosin pro-peptide such as chymosin (see Examples 1 and 2 of the application); red turnip beetle gut extract which comprises a mixture of proteases including aspartic proteases (see Example 3 of the application); pepsin and *Aspergillus saitoi* acid protease (Sigma 2143) as described in our amendment dated September 18, 2001. Consequently, we respectfully submit that with four examples of aspartic proteases that can cleave the chymosin pro-peptide from a heterologous polypeptide, it would be reasonable to predict that other aspartic proteases can also be used in this regard especially in view of the structural similarities of the aspartic proteases as described above.

With regard to the other conditions required in order to achieve cleavage, we submit that one of skill in the art could readily determine the conditions once they have selected a particular aspartic protease to be added to assist in the cleavage reaction. The optimum pH and other conditions required to achieve cleavage with various aspartic proteases are well known and readily available to one skill in the art.

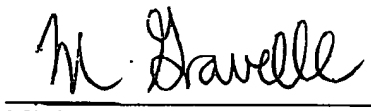
The Commissioner is hereby authorized to charge any deficiency in fees (including any claim fees) or credit any overpayment to our Deposit Account No. 02-2095.

Attached h reto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

In view of the foregoing, we submit that the claims (as amended herewith) are enabled by the specification and we ask that the objection to the claims under 35 USC §112, first paragraph be withdrawn.

Respectfully submitted,

Maurice Moloney, Joenel Alcantara and Gijs van Rooijen



Micheline Gravelle
Registration No. 40,261

Bereskin & Parr
Box 401, 40 King Street West
Toronto, Ontario, Canada
M5H 3Y2

(416) 957-1682

Version with markings to show changes made

Claims 1, 6, 13-16, 20, 25, 41, 42 and 44 have been amended as follows:

1. (Four Times Amended) A method for the preparation of a recombinant polypeptide comprising

a) transforming a host cell with an expression vector comprising:

(1) a nucleic acid sequence capable of regulating transcription in a host cell, operatively linked to

(2) a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a nucleic acid sequence encoding a chymosin pro-peptide [from an autocatalytically maturing aspartic protease], linked in reading frame to (b) a nucleic acid sequence heterologous to the pro-peptide and encoding the recombinant polypeptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide; operatively linked to

(3) a nucleic acid sequence encoding a termination region functional in said host cell,

b) growing the host cell to produce said fusion protein; and

c) adding a mature form of an autocatalytically maturing aspartic protease, that is capable of cleaving the chymosin pro-peptide, [zymogen] to the fusion protein so that the chymosin pro-peptide is cleaved from the fusion protein to release the recombinant polypeptide.

6. (Twice Amended) The method according to claim 1 wherein the chimeric nucleic acid sequence does not include a sequence encoding a mature form of [the aspartic protease] chymosin.

13. (Thrice Amended) A method according to claim 1 wherein the mature form of the [autocatalytically maturing zymogen] aspartic protease added in step (c) is [an aspartic protease] chymosin.

14. (Twice Amended) A method according to claim 1 wherein [the mature form of the autocatalytically maturing zymogen] the aspartic protease added in step (c) is heterologous to the chymosin pro-peptide.

15. (Amended) The method according to claim 13 wherein the [mature zymogen] chymosin is added under in vitro conditions.

16. (Amended) The method according to claim 13 wherein the [mature zymogen] chymosin is added under in vivo conditions.

20. (Twice Amended) A chimeric nucleic acid sequence encoding a fusion protein comprising (a) a nucleic acid sequence encoding a chymosin pro-peptide [from an autocatalytically maturing aspartic protease] and (b) a nucleic acid sequence encoding a polypeptide that is heterologous to the chymosin pro-peptide.

25. (Twice Amended) A chimeric nucleic acid sequence according to claim 20 which does not include a sequence encoding a mature form of [the aspartic protease] chymosin.

41. (Twice Amended) A composition comprising a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a first nucleic acid sequence encoding a chymosin pro-peptide [from an

autocatalytically maturing aspartic protease] and (b) a second nucleic acid sequence encoding a polypeptide that is heterologous to the chymosin pro-peptide.

42. (Twice Amended) A food composition comprising a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a first nucleic acid sequence encoding a chymosin pro-peptide [from an autocatalytically maturing aspartic protease] and (b) a second nucleic acid sequence encoding a polypeptide that is heterologous to the chymosin pro-peptide.

44. (Twice Amended) A composition according to claim 41 wherein said chimeric nucleic acid sequence does not include a sequence encoding a mature form of [the aspartic protease] chymosin.

Claim 23 has been deleted.